DIVISION : RESEARCH

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OBJECTIVE

To control the germination of bacterial spores during tobacco processing and the bacterial proliferation in the RL process in order to replace traditional preservative systems by a biocontrol agent.

STATUS

D-alanine, D,L-alanine, propyl paraben and decanoic acid were evaluated as inhibitors of B. pumilus spore germination. Germination of B. pumilus spores was studied in different tobacco extracts.

Trials to simulate SEL spoilage under the conditions of the RL process in the laboratory were performed.

RESULTS

Germination inhibitors

The efficiency of D-alanine, D,L-alanine, sodium propyl 4-hydroxybenzoate and decanoic acid as inhibitors of B. pumilus spore germination was investigated in ML cut filler and RL feedstock extracts incubated at 37°C. The germination was measured by loss of absorbance at 660 nm after inoculation by B. pumilus spore suspension [1].
The inhibitory activity of D-alanine in SEL (Fig. 1) was similar to that obtained previously in ML cut filler extracts [2]. The racemic mixture D,L-alanine appeared to be less efficient than the D-enantiomeric form (Fig. 2). After 24 hours incubation, however, bacterial growth was measured in most of the trials,

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even those treated with 900 ppm (10 mM) D- or D,L-alanine. Under these conditions, D-alanine appeared more as a germination retardant than a strict inhibitor.
The question of the efficiency of such systems in the RL process is raised. The answer requires simulation experiments under the process conditions and with the bacterial strains involved in SEL spoilage.
Figures 3 and 4 show propyl paraben and decanoic acid inhibition of B. pumilus spore germination on ML cut filler extract. The minimal inhibitory concentration (MIC) is between 100 and 200 ppm for propyl paraben and between 10 and 50 ppm for decanoic acid after 24 hours of incubation.

SEL simulation

Experiments were conducted to reproduce the SEL spoilage under lab conditions in order to isolate the specific contaminating microorganisms.
RL feedstock received from C Pilot Plant in Richmond was extracted (ratio 1:10) with de-ionised water at 80°C for 20 minutes. The fresh SEL was filtered into sterile flasks and incubated at 55°C and 120 rpm. Samples were collected over time, the pH measured and variations of bacterial populations were analysed. Concentration of isovaleric acid reported to be a good indicator of spoilage [3] was determined by GC [4].

Three different trials were performed. None showed any spoilage of the SEL after 30 days. Total number of bacteria and pH both decreased gradually. Concentration of isovaleric acid, not detected in the fresh extract, was about 3-6 ppm after 30 days.

Tobacco extracts

Burley, Flue-cured and Oriental tobaccos as well as ML cut filler and RL feedstock were water extracted (ratio 1:10) at 20°C as described previously [2].
B. pumilus spores were inoculated in the different extracts and the germination kinetics recorded during incubation at 37°C. In Flue-cured and Oriental extracts, germination did not occur even after 24 hours incubation (Fig. 5). Rapid germination kinetics were recorded in Burley and to a lesser extent in SEL and ML cut filler extracts. As nitrate in Burley can be considered as a germination activator [5], Flue-cured and Oriental extracts were supplemented with NO_3^- (0.1 - 0.5% w/v) and checked for germination. No positive effect could be recorded. This leads us to presume an inhibitory effect of Flue-cured and Oriental rather than an activating effect of nitrate in Burley extracts. This was confirmed by cross-mixing and diluting the different extracts and testing them for germination (Fig. 6).

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CONCLUSIONS

D- and D,L-alanine delay spore germination in ML cut filler extract and SEL, but are not absolute inhibitors.

Compounds present in Flue-cured and Oriental but not in the Burley extract seem to have inhibitory effects on germination.

No spoilage of the SEL could be simulated in lab scale experiments at 55°C.

PLANS

- Evaluate the effect of D-alanine on bacterial strains isolated in Richmond from spoiled SEL.
- Investigate the inhibitory effects of Flue-cured and Oriental tobaccos on B. pumilus spore germination.
- Compare the effects of different germination inhibitors in the SEL.
- Repeat the trials with propyl paraben and decanoic acid in order to confirm the exact MIC for germination.
- Test the stability of D-alanine over time in tobacco extract.

REFERENCES

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Fig. 1

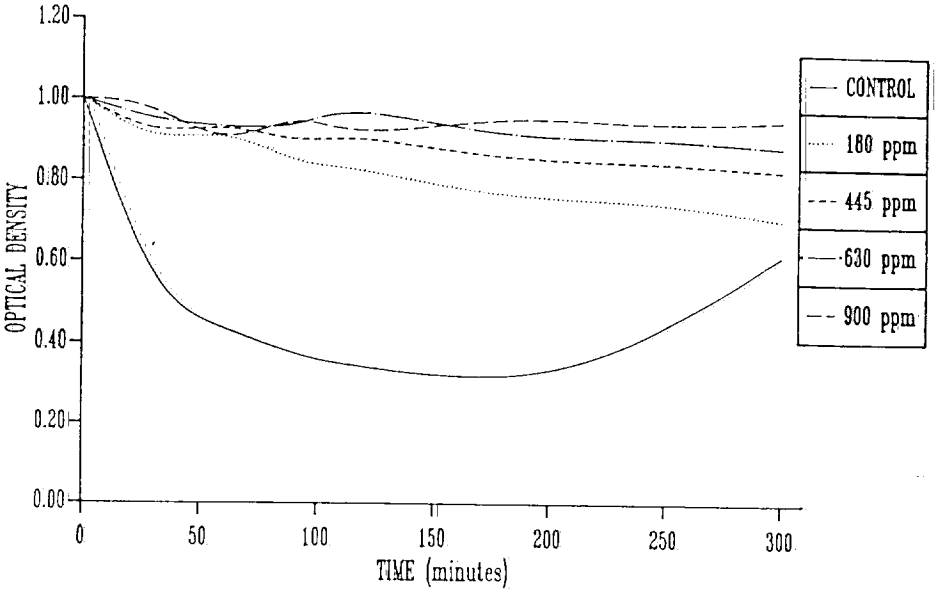

Fig. 2

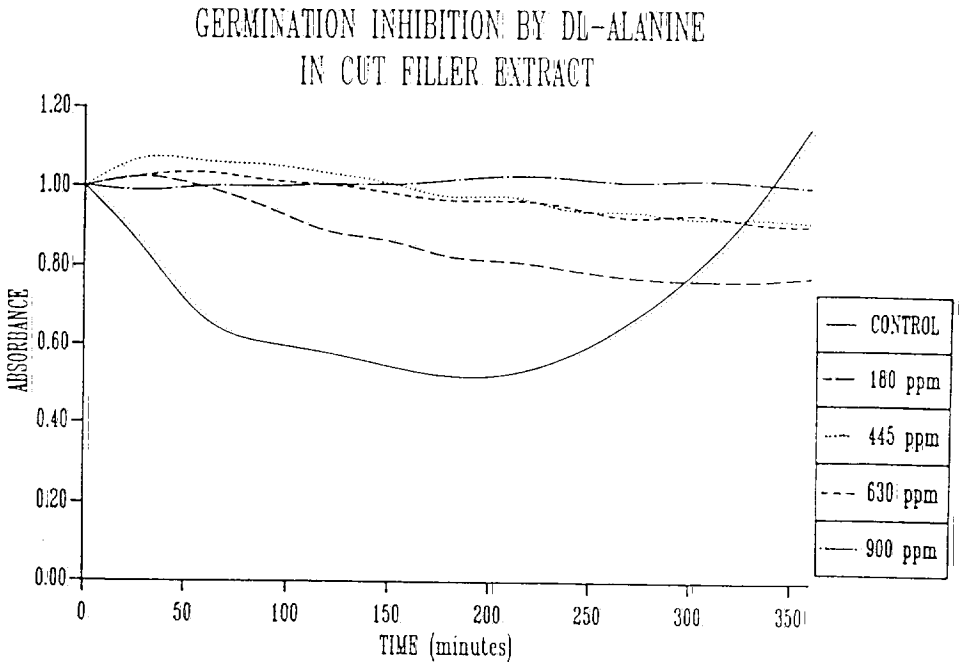

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Fig. 3

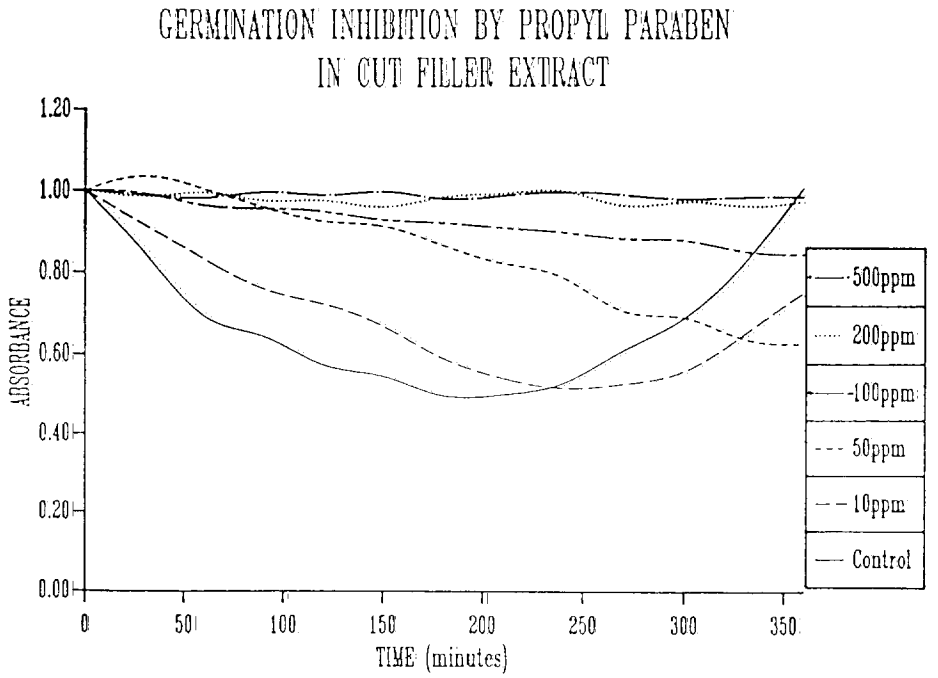

Fig. 4

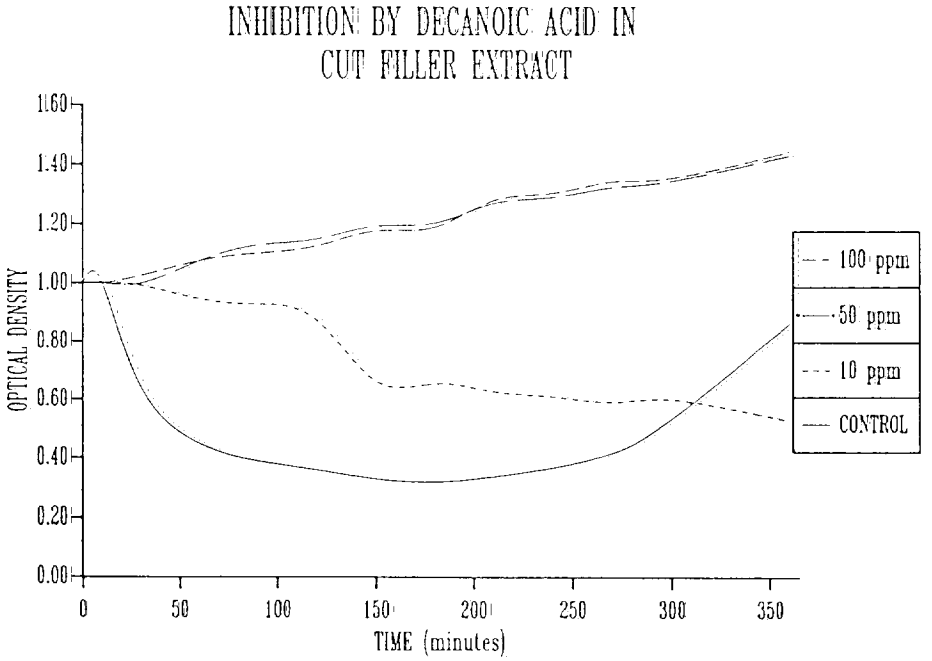

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Fig. 5

GERMINATION IN DIFFERENT TOBACCO EXTRACTS

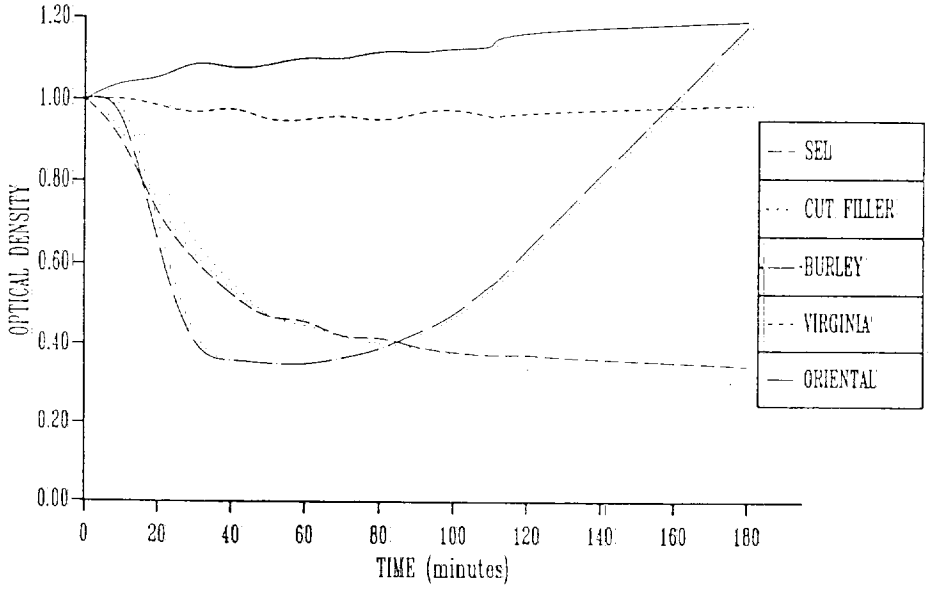
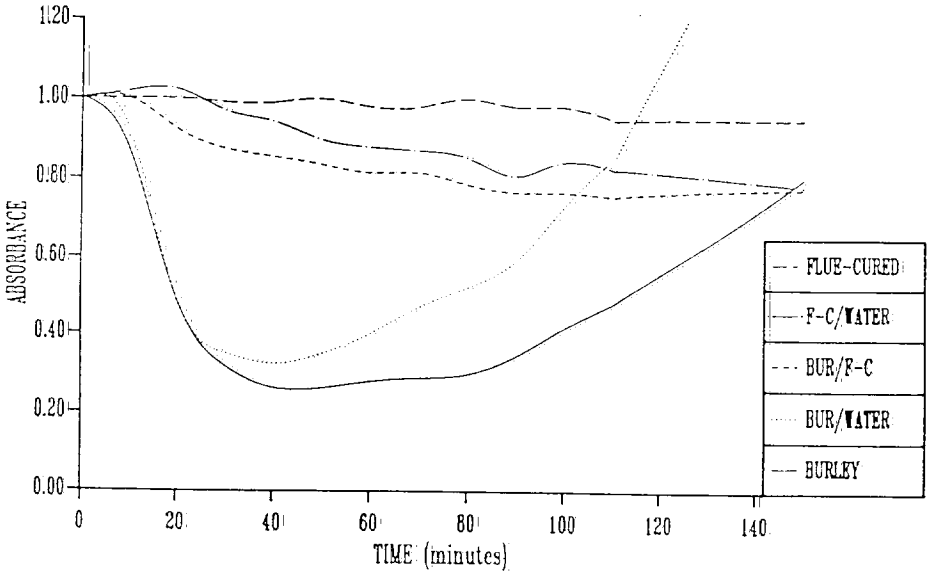


Fig. 6

GERMINATION IN DIFFERENT TOBACCO EXTRACTS



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